RECEIVED
CENTRAL FAX CENTER
AUG 0 2 2005

HUBR 1067.1 DIV. - PFF/SLH

IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

Applicants	:	Christoph SEIDEL et al.
Serial No.	:	08/892,704
Filed	:	July 15, 1997
For	:	RECOMBINANT ANTIGEN FROM THE NS3 REGION OF THE HEPATITIS C VIRUS
Art Unit	:	1643
Examiner	:	J. Williams

DECLARATION UNDER 37 C.F.R. § 1.132 OF URSULA-HENRIKE WIENHUES-THELEN

Ursula-Henrike Wienhues-Thelen, declares as follows:

(i) I am one of the co-inventors in the above-identified application. Attachment 1 is my *curriculum vitae*.

(ii) I am familiar with the present application, and I have read and understood this application.

(iii) I supervised certain experiments as described below and conclude as follows:

The results reported herein demonstrate that modifying the cysteine residues of the hepatitis C virus (HCV) polypeptide of the present invention as is taught in the present specification at pages 4-5 with a covalent modifying group (Example 1, discussed *infra*), or replacing the cysteine residues of the polypeptide of the present invention as is taught on page 5 of the specification with other natural or artificial amino acids (Example 2, discussed

infra), substantially increases the overall sensitivity of the method claimed in claims 29-32 by increasing the immunological reactivity of the polypeptide with HCV antibody. In addition, the results (Example 3, below) show that the concentration of releasable sulfhydryl groups of non-modified and modified HCV helicase antigen under reducing conditions may be readily determined.

Accordingly, the specification provides sufficient guidance to enable one skilled in the art to practice the invention claimed in claims 29-32 without the exercise of undue experimentation.

Methods and Materials

Example 1. Modification of the HCV antigen by Covalent Attachment of a Modifying Group

Cysteine residues of the HCV helicase antigen were modified by iodoacetate according to procedures well known in the art. The immunological reactivity of the modified HCV helicase antigen versus unmodified HCV helicase antigen with anti-HCV-positive human sera was determined using the double-antigen bridge test as set forth in Example 5 of the specification.

Results

As shown in Attachment 2, the covalently modified HCV helicase antigen was more specific for the antibodies in anti-HCV-positive human serum compared to the unmodified HCV helicase antigen. Accordingly, the modification of cysteine residues at HCV polypeptides considerably increased the overall sensitivity of a method for detecting anti-hepatitis C virus antibodies.

Example 2. Modification of the HCV Antigen by Replacement of Cysteine Residues with Another Amino Acid.

In this experiment, cysteine residues of the HCV helicase antigen were substituted for serine residues by site-specific mutagenesis. The reactivity of modified versus unmodified HCV helicase antigen with anti-HCV-positive human sera was determined using the double antigen bridge test as set forth in Example 5 of the specification.

Results

As shown in Attachment 3, the mutagenized HCV helicase antigen was more specific for the antibodies in the serum compared to the unmodified antigen. Accordingly, the polypeptides with substituted cysteine residues increased the overall sensitivity of a method for detecting anti-hepatitis C virus antibodies.

Example 3. Method of Determining the Concentration of Releasable Sulfhydryl Groups

An aliquot of the helicase to be examined is mixed with 20 mM DTT and incubated at 37°C for 1 hour.

The reducing agent is separated off by means of chromatography using Sephadex G-25 or by dialysis against 0.1 M sodium phosphate buffer, pH 6.0, 0.1% SDS.

0.25 mg helicase are then diluted to 1 ml with 0.15 M sodium phosphate buffer, pH 7.6, 2mM EDTA and mixed with 0.03 ml of a DTDP solution (11 mg dithiodipyridine dissolved in 5 mM sodium phosphate buffer, pH 6.0, 1mM EDTA).

The mixture is incubated at 25°C for 2 minutes, then the extinction is photometrically measured at 334 nm. The extinction is corrected by the reagent blank value (DTDP in

sample buffer without helicase) and the sample blank value (helicase in sample buffer without DTDP).

The concentration of the sulfhydryl groups is then computed by means of Lambert-Beer's Law (ε_{324nm} = 15.2 cm x $mmol^{-1}$).

Results

Attachment 4 shows the concentration of releasable sulfhydryl groups of modified and non-modified HCV helicase antigen under reducing conditions. Cysteine-modified antigens contain less sulfhydryl groups which are releasable under reducing conditions.

In conclusion, the above results demonstrate that the modification of cysteine residues by covalent attachment of a modifying group or replacement of cysteine residues by another amino acid, significantly increases the sensitivity of the method for detecting HCV antibody as claimed in claims 29-32, as evidenced by the increased immunological reactivity of modified helicase antigen with anti-HCV-positive human sera.

I hereby declare that all statements made herein of my own knowledge are true and that all statements made on information and belief are believed to be true; and further that these statements were made with the knowledge that willful false statements and the like so made are punishable by fine or imprisonment, or both, under Section 1001 of Title 18 of the United States Code and that such willful false statements may jeopardize the validity of the application or any patent issued thereon.

5.5.1998
Date

Ursula-Henriette Graeber

CURRICULUM VITAE

name:	Ursula-Henrike Wienhues-Thelen
day of birth:	15.09.1959
place of birth:	Cologne
1965-1969:	primary school in Cologne
1969-1978:	secondary school in Cologne
16.june 1978:	final examination
october 1978:	start with the study of biology at the university of Cologne
03.october 1980:	„Vordiplom“
march-april 1982:	research work with professor K. Willecke at the institute of cellbiology at the university, Uniklinikum Essen
jan. - sept. 1983:	research work with professor W. Doerfler at the institute of genetics in Cologne
31.october 1984:	finishing diploma thesis in professor W.Doerfler´s laboratory
march - may 1987:	research work with professor K.Hosokawa at the Kawasaki Medical School in Okayama / Japan
07.may 1988:	finishing doctor thesis in professor W.Doerfler´s laboratory
1988 - 1991:	postdoc in professor W.Neupert`s laboratory in the Institut für Physiologische Chemie in Munich
since september 1991:	development of diagnostica, Boehringer Mannheim GmbH, Tutzing

München,
16.03.1998 Ursula-Henrike Wienhues-Thelen

ATTACHMENT 2

Example regarding covalently modified cysteines

Evaluation of the reactivity of differently modified helicase antigens with anti-HCV-positive human sera

	Reactivity of helicase antigen, without chemical modification of the cysteines cut off index	Reactivity of helicase antigen the cysteines of which are chemically modified with a protective group: iodine acetate (IAA) cut off index
Serum 1	20.9	33.2
Serum 2	15.1	24.4
Serum 3	4.0	14.5

Carried out in analogy to Example 5.

ATTACHMENT 3

Example regarding the replacement of cysteines by other amino acids

Evaluation of the reactivity of differently modified helicase antigens with anti-HCV-positive human sera

	Reactivity of helicase antigen, without chemical modification of the cysteines cut off index	Reactivity of helicase antigen having mutagenized cysteines (all but two cys residues are serine) cut off index
Serum 1 (early seroconversion)	0.9	11.3

ATTACHMENT 4

SH status of rec. HCV helicase, HCV helicase mutein and derivatives

Helicase	Modification	SH groups releasable under reducing conditions
HCV helicase, underivatized	none	6.4
HCV helicase, biotinylated	chemically with IAA	2.8
HCV helicase, ruthenylated	chemically with IAA	3.8
HCV helicase mutein	cysteine replacement	1.9
HCV helicase mutein, biotinylated	cysteine replacement	0.15
HCV helicase mutein, ruthenylated	cysteine replacement	0.6